Indian J Med Res 88, September 1988, pp 211-216

Intraspecies differentiation of strains of *Mycobacterium tuberculosis* obtained from Czechoslovakian, Mongolian and South Indian patients

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Accepted June 3, 1988

Twenty nine strains of *M. tuberculosis* from Czechoslovakia, 46 from Mongolia and 50 from South India were tested for virulence in the guineapig, sensitivity to thiophen-2-carboxylic acid hydrazide (TCH) and phage type. Most of the Czechoslovakian and Mongolian strains (93 and 80% respectively) were highly virulent in the guineapig while only 36 per cent of the South Indian strains showed high virulence. Similarly very high proportions of Czechoslovakian (97%) and Mongolian strains (85%) were resistant to TCH as against only 22 per cent of South Indian strains. The phage type I was observed in none of the Czechoslovakian strains, 4 per cent of Mongolian strains and in 68 per cent of South Indian strains. Thus, the Czechoslovakian and Mongolian strains, in general, resembled the classical M. tuberculosis, while the South Indian strains were generally of low virulence, susceptible to TCH and of phage type I.

Evidence accumulated over the last two decades has shown that there exist within the species *Mycobacterium tuberculosis*, some variants and types on a subspecies level. The most prominent of these, the South Indian variant of *M. tuberculosis*, is distinguished by a number of characteristic features *viz.*, lower virulence in the guineapig, enhanced susceptibility to hydrogen peroxide and thiopen-2-carboxylic acid hydrazide (TCH), natural resistance to thioacetazone and p-amino salicylic acid, clustering into an intermediate phage type I and characteristic composition of cell wall lipids[1-5]. However, very little information about comparative characteristics of strains from different countries is available.

Comparative characteristics obtained in a study of strains of *M. tuberculosis* isolated from patients in Mongolian People's Republic, Czechoslovakia and the Chingleput District of South India (BCG trial area) which were tested for three main characteristics-namely, virulence in the guineapig, mycobacteriophage type and susceptibility to thiophen-2-carboxylic acid hydrazide, is presented in this report.

Material & Methods

Strains: Twenty nine strains from Czecho-Slovakia and 46 from Mongolia were obtained through Dr Milan Kubin as part of an International Collaborative Investigation. In addition, 50 strains obtained from the Chingleput District of South India were also included.

Sensitivity to isoniazid: The strains were tested for their sensitivity to isoniazid in on Lowenstein-Jensen (L–J) medium containing 0.2, 1 and 5 mg/l. A minimal inhibitory concentration (MIC) of 5 mg/l was considered as resistant.

Virulence tests: Virulence studies in the guineapig were done using the methods described by Mitchison *et al* [1]. In brief, for each strain, a pair of guineapigs was injected with a suspension containing 1 mg (moist weight) of the organism. The animals were coded and sacrificed at the end of 6 wk and the extent of visible tuberculous lesions in the spleen, liver, lungs and lymph nodes was scored. The square root of the ratio of the total score to the survival period in days was expressed as the Root Index of Virulence (RIV). An RIV of 1.0 or more was taken as indicative of high virulence while an RIV of less than 1.0 was considered to be indicative of low virulence.

Sensitivity to thiophen-2-carboxylic acid hydrazide: TCH was incorporated in L-J medium to give final concentrations of 0.5, 1.0, 2.5, 5, 10 and 20 mg/l. A standard suspension of the organism was prepared so as to contain approximately 4 mg/ml and a loopful (3mm diameter, 27 SWG) of this was inoculated onto each of the slopes. The slopes were read after 4 wk of incubation and the minimal inhibitory concentration (MIC) determined. An MIC of 20 mg/l or more was indicative of resistance to the compound[4].

Mycobacteriophage typing: Phage types were determined using the definitions of Grange *et a1* [4]. The phages MTPH 1, 2, 3, 4, 5 and 9 were obtained from Dr H.B.W. Engel of the Netherlands and propagated in this laboratory. Using these phages the *M. tuberculosis* strains can be broadly divided into 3 main types-A, B and I as indicated Table 1.

Results

Isoniazid sensitivity: Of the 46 Mongolian strains, 18 (39%) were found to be isoniazid resistant. In contrast, none of the Czecho-slovakian or South Indian strains were resistant.

Root index of virulence: The distribution of strains from the 3 regions according to the RIV is presented in Table II. Most (64%) South Indian isolates were of low virulence in the guineapig when compared to

Table I. Classification of phage types

Phage type	MTPH					
	1	2	5	9	4	3
A	+	+	–	–	–	–
I	+	+	+	–	–	–
	+	+	+	+	–	–
	+	+	+	+	+	–
B	+	+	+	+	+	+

+: lysis by phage at routine test dilution
–: no lysis

Czechoslovakian (7%) and Mongolian (20 %) cultures, a highly significant difference (P<0.001). All the 9 low virulent strains from Mongolia were isoniazid resistant.

Phage types: Among the 29 Czechoslovakian strains, 23 (79%) belonged to type A and 6 (21%) to type B. No strain of the I type was seen. Of the 46 Mongolian strains, 39 (85 %) belonged to type A and 5 (11%) to Type B; only 2 (4%) were of type I.

Among the South Indian strains, 34 (68 %) were of type I while 16 (32 %) belonged to type A. Type B was absent in this group. Thus, while the Czechoslovakian and Mongolian strains were predominantly of the A type, the majority of the South Indian strains were of the I type.

TCH susceptibility: Only one (3%) of the 29 Czechoslovakian strains was sensitive to TCH (Table III). Among the Mongolian strains 15 per cent were sensitive to TCH while 85 per cent were resistant. In the South Indian strains, 78 per cent were sensitive, 42 per cent among them having an MIC of 2.5 mg/l or less.

Table II. Distribution of strains from the three regions according to the root index of virulence (RIV)

RIV	Czechoslovakian		Mongolian		S. Indian	
≤ 0.5	0		0		1	
0.51-0.74	1	7 %	4	20%	18	64%
0.75-0.99	1		5		13	
1.00-1.19	2		7		10	
1.20-1.39	21	93%	19	80%	7	36%
≥ 1.40	4		11		1	

Table III. TCH susceptibility patterns of strains

TCH MIC	Czechoslovakian		Mongolian		S. Indian	
≤ 2.5	0		0		21	
5	0	3%	4	15%	10	78%
10	1		3		8	
≥ 20	28	97%	39*	85%	11	22%
Total	29		46		50	

*Includes 18 INH-resistant strains

Discussion

Intraspecies variations in *M. tuberculosis* have been known for nearly two decades. As early as 1948, Dhayagude and Shah[6] showed that the Indian strain of tubercle bacilli was attenuated in the guineapig as compared with the classical human type of *M. tuberculosis.* Other tests used to characterise this strain include hydrogen peroxide susceptibility[2,7] susceptibility to PAS and thioacetazone[3], phage typing[4,5,8] and cell wall lipids[5,9]. This variant, known as the Asian type of *M. tuberculosis* has been considered to have evolved as a result of parallel and divergent evolution in geographically distinct regions[5]. Similar intraspecies variations have also been reported in other mycobacterial species like *M. chelonei* [10,11] and *M. kansasii* [12].

In the present report we have studied the three main characteristics of *M. tuberculosis* strains obtained from Czechoslovakia, Mongolia and South India. The Mongolian strains came from an ethnically homogenous population of low migration activity,

whereas, the other two groups came from East Europe and South East Asia, and in terms of their properties represented the 'classical' *M. tuberculosis* and its South Indian variant.

Considering the phage types, strains from Czechoslovakia and Mongolia were similar. The A and B types accounted for virtually all strains, only 2 out of 75 strains belonging to I type. In striking contrast, 68 per cent of the South Indian strains were of the I type. This is in agreement with other reports from Madras[8] and Nepal[13] on phage types (Table IV).

As regards susceptibility to TCH, all but one of the Czechoslovakian strains were resistant to this compound. Even this odd culture had an MIC of 10, which could be regarded as of borderline resistance. Thus, this group exhibited the characteristics of class&al human strains. Among the Mongolian strains, 15 per cent were sensitive, again including 7 per cent with borderline resistance.

Of the South Indian strains, 78 per cent were sensitive to TCH; there was a wide variation in the MIC levels, 42 per cent having an MIC of 2.5 or less. On the other hand, 33 of 34 strains obtained from the Kashmir valley region in North India were resistant to TCH[14]. Strains isolated in Nepal showed 100 per cent resistance to furan-2-carboxylic acid hydrazide, a compound similar to TCH[13]. Thus, TCH sensitivity seems to be a characteristic feature of the South Indian strains.

Of the 39 resistant strains from Mongolia, as many as 18 were INH resistant. It

Table IV. Phage types of *M. tuberculosis* in different geographical regions

Country/region	Phase type (%)			Reference
	A	B	I	
U.S.A.	76	14	–	Bates and Fitzhugh[16]
Madras	54	13	43	Bates and Mitchison [8]
Hong Kong	89	5	6	
Rhodesia	78	–	22	
British	50	45	5	
British	66	30	4	Grange *et al* [17]
Asian	66	6	28	
Uganda	88	3	9	Grange *et al* [4]
Brent Asians	63	9	28	
Kashmir	91	6	3	Mayurnath *et al* [14]
Nepal	19	28	53	Goode[13]
Czechoslovakia	79	21	–	Present report
Mongolia	85	11	4	
South India	32	–	68	

is well known that INH and TCH, having a chemically similar structure, exhibit cross-resistance *in vitro*. This fact should be taken into consideration while interpreting any results on TCH resistance. However, a recent report by Yates *et al* [15] has claimed that this cross-resistance is not a universal event.

Considering the virulence of these strains in the guineapig, most of the Czechoslovakian strains had a high RIV, again resembling the classical *M. tuberculosis.* In the Mongolian group, 9 had low virulence, all 9 being INH resistant. However, 9 other strains which were resistant to INH, showed a high virulence although isoniazid resistant strains are known to be attenuated in the guineapig. Goode[13] has reported that INH-resistant strains isolated from patients in Nepal were virulent in the guineapig and that isoniazid resistance is not always a reliable marker of lower virulence in guineapigs.

Considering all the three characteristics together, the Czechoslovakian and Mongolian strains, in general, resembled the classical *M. tuberculosis,* while the South Indian strains were generally of low virulence in the guineapig, susceptible to TCH and were predominantly of phage type I. These properties have been fairly stable over the last two decades and could be considered as a distinct feature of the South Indian isolates of *M. tuberculosis.*

Acknowledgment

The authors thank Dr Milan Kubin, Institute of Hygiene and Epidemiology, Prague, Czechoslovakia for providing the strains used in this investigation, and Dr H.B. W. Engel, Rijks Institute Voor Volksgezondheid en milienhygiene, Bilthoven, Netherlands, for supplying the phages and host strains. The authors also thank Dr S.P. Tripathy, Additional Director-General, Indian Council of Medical Research, New Delhi, for his keen interest in the conduct of the study.

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